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Adenosine A_{2A} receptor antagonists are potential antidepressants: evidence based on pharmacology and A_{2A} receptor knockout mice

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1 Adenosine, an ubiquitous neuromodulator, and its analogues have been shown to produce 'depressant' effects in animal models believed to be relevant to depressive disorders, while adenosine receptor antagonists have been found to reverse adenosine-mediated 'depressant' effect.

2 We have designed studies to assess whether adenosine A_{2A} receptor antagonists, or genetic inactivation of the receptor would be effective in established screening procedures, such as tail suspension and forced swim tests, which are predictive of clinical antidepressant activity.

3 Adenosine A_{2A} receptor knockout mice were found to be less sensitive to 'depressant' challenges than their wildtype littermates. Consistently, the adenosine A_{2A} receptor blockers SCH 58261 (1–10 mg kg^{-1}, i.p.) and KW 6002 (0.1–10 mg kg^{-1}, p.o.) reduced the total immobility time in the tail suspension test.

4 The efficacy of adenosine A_{2A} receptor antagonists in reducing immobility time in the tail suspension test was confirmed and extended in two groups of mice. Specifically, SCH 58261 (1–10 mg kg^{-1}) and ZM 241385 (15–60 mg kg^{-1}) were effective in mice previously screened for having high immobility time, while SCH 58261 at 10 mg kg^{-1} reduced immobility of mice that were selectively bred for their spontaneous 'helplessness' in this assay.

5 Additional experiments were carried out using the forced swim test. SCH 58261 at 10 mg kg^{-1} reduced the immobility time by 61%, while KW 6002 decreased the total immobility time at the doses of 1 and 10 mg kg^{-1} by 75 and 79%, respectively.

6 Administration of the dopamine D_2 receptor antagonist haloperidol (50–200 $\mu g\ kg^{-1}$ i.p.) prevented the antidepressant-like effects elicited by SCH 58261 (10 mg kg^{-1} i.p.) in forced swim test whereas it left unaltered its stimulant motor effects.

7 In conclusion, these data support the hypothesis that A_{2A} receptor antagonists prolong escape-directed behaviour in two screening tests for antidepressants. Altogether the results support the hypothesis that blockade of the adenosine A_{2A} receptor might be an interesting target for the development of effective antidepressant agents.

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Keywords: Adenosine; A_{2A} receptor; A_{2A} receptor knockout mice; antidepressant; forced swim test; tail suspension test; motor activity; SCH 58261; KW 6002; ZM 241385

Abbreviations: DMSO, dimethyl sulphoxide; KW 6002, (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine; SCH 58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; ZM 241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-yl-amino]ethyl)phenol

Introduction

There is evidence that adenosine is a neuromodulator which takes part in a variety of processes in both physiological and pathological conditions. In the central nervous system, adenosine is involved in controlling behavioural states along the continuum wakefulness-sedation (Porkka-Heiskanen, 1999), has been associated with mood changes such as anxiety (Jain *et al.*, 1995; El Yacoubi *et al.*, 2000a), is involved in cognitive processes (Kopf *et al.*, 1999) and has an important role in the regulation of motor activity (Brockwell & Beninger, 1996). Research efforts made over the last 20 years have resulted in the discovery of four G-protein coupled receptors which specifically bind adenosine to produce biological effects (Olah & Stiles, 2000). These receptors, namely adenosine A_1, A_{2A}, A_{2B} and A_3 receptors, have distinct distributions and control different functions in the mammalian organism. In the brain, adenosine A_1 receptors are abundant, especially in the cortex, whereas A_{2A} receptors are mainly located in the striatum. Conversely, both adenosine A_{2B} and A_3 receptors are present in low amounts in the brain. The A_{2B} receptor has recently been shown to constitute also a receptor for the neurotrophic factor netrin-1 (Corset *et al.*, 2000), while the function of the A_3 receptor remains to be elucidated (Impagnatiello *et al.*, 2000).

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A variety of studies have shown that blocking the A_{2A} receptors leads to significant improvement of motor dysfunction. Among potent adenosine A_{2A} receptor antagonists used as tools in these pharmacological studies, SCH 58261 showed an excellent selectivity profile at human adenosine receptors in a recent study (Ongini *et al.*, 1999): A_{2A} (Ki 0.6 nM)<A_1 (Ki 287 nM)<A_{2B} (Ki 5,011 nM)<A_3 (Ki>10,000 nM). In the same study, ZM 241385, another potent non-xanthine A_{2A} receptor antagonist (Ki 0.8 nM), also showed little affinity for A_1 receptor (Ki 255 nM) and did not interact with A_3 (Ki>10,000 nM); however, it displayed moderate affinity for A_{2B} receptors (Ki 50 nM). This less favourable profile has been confirmed later (Klotz, 2000). The xanthine-like derivative KW 6002 was shown to display high affinity for A_{2A} receptor (Ki 2.2 nM), moderate A_{2A} versus A_1 selectivity and to be active in experimental models of Parkinson's disease (Shimada *et al.*, 1997; Shiozaki *et al.*, 1999). Hence, adenosine A_{2A} receptor antagonists are considered as potential drugs in the treatment of movement disorders such as Parkinson's disease (Ongini & Fredholm, 1996; Richardson *et al.*, 1997). This activity is believed to depend upon the close anatomical and functional association between adenosine A_{2A} and dopamine D_2 receptors on the so-called indirect striato-pallidal GABAergic pathway (Ferré *et al.*, 1997). Thus, blockade of the adenosine A_{2A} receptors would reinstate normal movements through interactions with dopamine-mediated activity in basal ganglia.

Consistent with pharmacological data, genetic inactivation of the adenosine A_{2A} receptor gene has shown that knockout mice are more resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin which produces damage similar to that observed in Parkinson's disease (Chen *et al.*, 2000).

Other data suggest that A_{2A} receptors are involved in mediating the effects of adenosine on behavioural states. Adenosine A_{2A} receptor knockout mice display behavioural changes, such as aggressiveness and hypoalgesia (Ledent *et al.*, 1997). In general, adenosine and its analogues tend to produce 'depressant' effects in animal models, believed to be relevant to human conditions. For example, stimulation of adenosine receptors or increase of adenosine levels induce a state of 'learned helplessness' similar to that observed in an animal model of depression generally considered as reliable (Minor *et al.*, 1994; Woodson *et al.*, 1998). Adenosine and 2-chloroadenosine increase the immobility time in the forced swim test in mice, a widely used model of depression (Porsolt *et al.*, 1977), while classical antidepressants have been found to reverse adenosine-mediated immobility (Kulkarni & Mehta, 1985).

The adenosine A_{2A} receptors might be involved in these processes through their interaction with dopamine D_2 receptors in the striatum, which, together with the dopamine neuronal transporters, are increased in depressed patients (D'haenen & Bossuyt, 1994; Shah *et al.*, 1997; Lansonen-Balk *et al.*, 1999). Consistent with these data, studies have shown that bromocriptine (Colonna *et al.*, 1979; Sitland-Marken *et al.*, 1990) and piribedil (Post *et al.*, 1978; Mouret *et al.*, 1987), two dopamine D_2 receptor agonists, which are mainly used for treatment of Parkinson's disease, show some antidepressant activity. Therefore, adenosine A_{2A} receptor antagonists, by acting on various circuitries in the brain, or more specifically by modulating mesostriatal or mesocorticolimbic dopaminergic pathways, may also possess antidepressant properties.

Within this background we have designed studies to assess whether adenosine A_{2A} receptor antagonists or genetic inactivation of the receptors, using adenosine A_{2A} receptor knockout mice, would be effective in established models of depression. The data show that reference adenosine A_{2A} receptor blockers produce dose-related effects in mouse models of depression such as the forced swim or the tail suspension tests. Consistently, adenosine A_{2A} receptor knockout mice were found to be less sensitive to 'depressogenic' challenges than their wildtype littermates. Altogether, the data support the hypothesis that blockade of the adenosine A_{2A} receptors might be an interesting and novel approach in the search of effective antidepressant agents.

Methods

Animals

Male Swiss albino CD1 mice bred by Charles River (Saint Aubin lès Elbeuf, France, and Calco, Italy), male Swiss albino CD1 mice selectively bred in our facilities (UMR CNRS 6036, Rouen, France) for high spontaneous 'helplessness' in the tail suspension test (Vaugeois *et al.*, 1996), or adenosine A_{2A} receptor knockout mice and their wildtype controls bred on a CD1 background for five to ten generations (Ledent *et al.*, 1997), weighing 20–30 g were used after at least one week of habituation in our own facilities. Mice were housed in groups of 15–20 in Makrolon cages (38×24×18 cm) with free access to water and food (U.A.R., France, and Charles River, Calco, Italy) and kept in a ventilated room at a temperature of 21°C±1°C, under a 12 h light/12 h dark cycle (light on between 0700 and 1900). Experiments were carried out between 0900 and 1900. The animals were isolated in small individual cages (27×13×13 cm) for 30 min prior testing.

The procedures described comply with ethical principles and guidelines for care and use of laboratory animals adopted by the European Community, law 86/609/CCE.

Tail suspension test

The tail suspension test is based on the observation that a mouse suspended by the tail shows alternate periods of agitation and immobility (Stéru *et al.*, 1985). The mouse, acoustically and visually isolated, was hung on the hook by an adhesive tape placed 20 mm from the extremity of its tail and it was kept 150 mm away from the nearest object. The sum of immobility periods (duration of immobility) was measured by an observer who was unaware of the drug treatments or by a computerized device (ITEMATIC-TST) developed by ITEM-LABO (Le Kremlin-Bicêtre, France). In the latter experimental condition, a strain gauge picked up all movements of the mouse and transmitted them to a central unit which calculated the total duration of immobility during a 6-min test (Stéru *et al.*, 1987). Using the computerized system, six animals could be tested at one time. Each mouse was used only once for each experimental session.

Forced swim test in mice

Mice were dropped individually into glass cylinders (height: 25 cm, internal diameter: 10 cm) containing 10 cm water, maintained at 23–25°C. The apparatus consisted of two Plexiglass cylinders placed side by side in a Makrolon cage (38 × 24 × 18 cm). Two mice were tested simultaneously for a 6-min period but a non-transparent screen placed between the two cylinders prevented mice from seeing each other. The immobility time was measured during the last 3 or 4 min of the test by an observer who was unaware of the drug treatment. A mouse was judged to be immobile when it remained floating in the water, making only the necessary movements to keep its head above water. Each mouse was used only once for each experimental session.

Reserpine test

Mice were given reserpine (2 mg kg^{-1} s.c.). A score of ptosis was measured for each eye 3.5 h later, as 0 (eye completely open) to 4 (eye fully closed), i.e., a maximum score of 8 per mouse. The rectal temperature was also measured 3.5 h later with a thermistor probe (Physitemp TH5, probe RM6; Clifton, U.S.A.) inserted to a depth of 2.5 cm into the rectum. The mice were then divided into vehicle and test drug groups and were introduced immediately after into the actimeters for a 30-min test session. Ptosis and rectal temperature were measured again at the end of the motor activity test.

Locomotor activity

Locomotor activity was measured with a Digiscan Animal Activity Monitor system (Omnitech Electronics Inc., Columbus, OH, U.S.A.) which monitored the horizontal (locomotion) and vertical (rearing) movements of the animals. The Digiscan analyser was interfaced with an IBM-PC compatible computer using Digipro software. The individual compartments (L = 20; W = 20; H = 30 cm) were put in a dimly lit and quiet room. Horizontal movements, i.e., locomotion, were expressed as number of beams crossed over two (experiment with reserpine) or three 15 min periods of testing.

Drugs

SCH 58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine, and KW 6002, (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine, were synthesized at the Schering-Plough Research Institute, Kenilworth, NJ, U.S.A. ZM 241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-yl-amino]ethyl)phenol was a generous gift from Dr S. Poucher (Zeneca Pharmaceuticals, Macclesfield, U.K.). SCH 58261 (1, 3, 10 mg kg^{-1}) and ZM 241385 (15, 30, 60 mg kg^{-1}) were dissolved in dimethyl sulphoxide (Sigma Chemical Co., St Louis, MO, U.S.A.) and then diluted in Cremophor EL (Sigma Chemical Co., St Louis, MO, U.S.A.) and NaCl 0.9% (final concentration: 15% DMSO and 15% Cremophor EL). In another set of experiments, SCH 58261 (1, 3, 10 mg kg^{-1}) and KW 6002 (1, 3, 10 mg kg^{-1}) were dissolved in a suspension vehicle (methyl cellulose 0.4%, tween 80, 0.5%, benzyl alcohol, 0.8% in saline). Reserpine (Sigma Chemical Co., St Louis, MO, U.S.A.) was dissolved in distilled water containing 5% dimethyl sulphoxide and 5% Cremophor EL (Sigma Chemical Co., St Louis, MO, U.S.A.) and injected s.c. haloperidol (Haldol®, Janssen, France) was diluted in saline in order to get the appropriate doses and administered by the i.p. route. Drug solutions were prepared fresh daily in a volume of 10 ml kg^{-1}. Doses always refer to the free bases.

Statistics

Results are expressed as means ± s.e.mean. Differences between means were analysed by Student's *t*-test or ANOVA (with one or two factors and with or without repeated measures where appropriate). Where *F* ratios were significant, multiple comparisons were evaluated by the Newman-Keuls multiple comparison test. Significance levels were set at $P < 0.05$.

Results

Response of adenosine A_{2A} receptor knockout mice in tail suspension and forced swim test

In the tail suspension test, the duration of immobility was reduced by 30% ($P < 0.05$) in adenosine A_{2A} receptor knockout mice as compared to wildtype animals (Figure 1A). Similarly, in the forced swim test, A_{2A} receptor knockout animals behaved differently from the wildtype mice as their time of immobility was reduced by 24% ($P < 0.001$) as compared to controls (Figure 1B).

Effects of adenosine A_{2A} receptor antagonists in the tail suspension test in CD1 mice

SCH 58261 (1, 3, 10 mg kg^{-1}, i.p.) dose-dependently reduced the immobility time by 51, 86 and 92%, respectively (Figure 2A). Similarly, another adenosine A_{2A} receptor antagonist, KW 6002 (0.1, 1, 10 mg kg^{-1}, p.o.) dose-dependently decreased the total immobility time, after oral administration, by 40, 74 and 91%, respectively (Figure 2B).

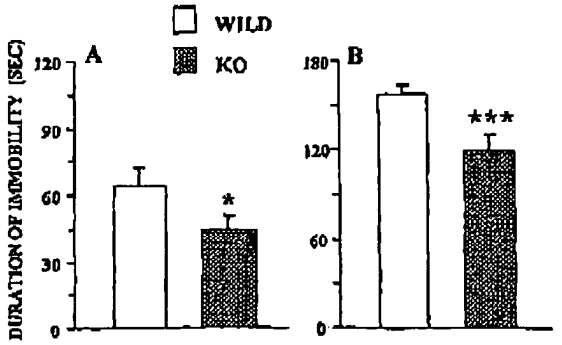

Figure 1 Immobility times of A_{2A} receptor knockout (A_{2A}R KO) and wildtype (A_{2A}R WT) mice recorded in the tail suspension or forced swim tests. (A) duration of immobility in the tail suspension test. Means ± s.e.mean of data from 29 mice per group. B: Duration of immobility in the forced swim test. Means ± s.e.mean of data from 16 mice per group. $^{*}P < 0.05$, $^{***}P < 0.001$ as compared to wildtype mice by Student's *t*-test.

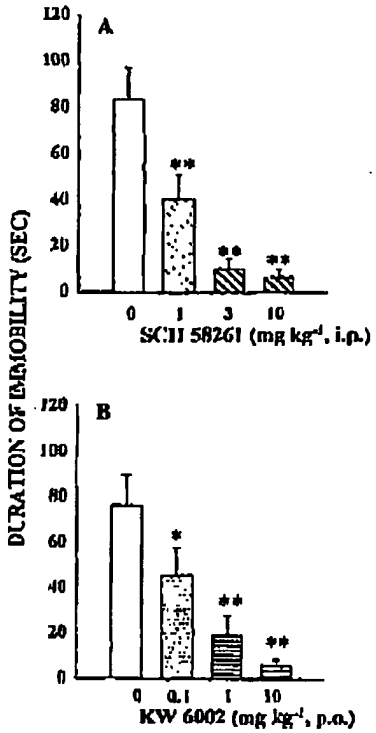

Figure 2 Effects of SCH 58261 (A) and KW 6002 (B) in the mouse tail suspension test. Mice were injected with vehicle or SCH 58261 (1, 3, 10 mg kg $^{-1}$, i.p.) 30 min before the test; or received vehicle or KW 6002 (1, 3, 10 mg kg $^{-1}$, p.o.), 60 min before the test. Data are mean + s.e.mean of 10 animals per group. $^{**}P<0.01$ versus vehicle-treated group by one-way ANOVA followed by Student-Newman-Keuls test.

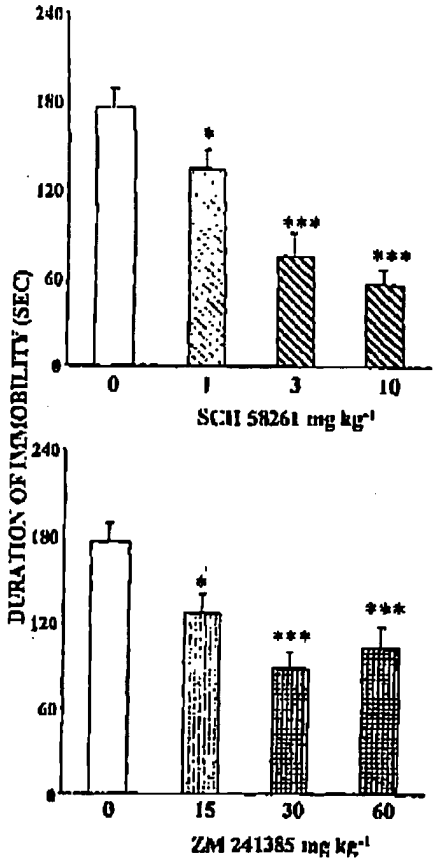

Figure 3 Effects of SCH 58261 and ZM 241385 in tail suspension test in screened male CD1 mice. Pre-test selection consisted of one trial on two consecutive days. For each selected 'high-immobility' mouse (III), the mean score was calculated and used as the pretest score. On day 3, mice were injected with vehicle, SCH 58261 (1, 3, 10 mg kg $^{-1}$ i.p.) or ZM 241385 (15, 30, 60 mg kg $^{-1}$ i.p.) 30 min before the test. Means + s.e.mean of data from 17 controls and 13–15 mice in treated groups. $^{*}P<0.05$, $^{***}P<0.001$ (one-way ANOVA followed by Student-Newman-Keuls test).

Under a repeated treatment schedule (3 mg kg $^{-1}$ i.p., twice daily for 8 days), SCH 58261 decreased by 44% the duration of immobility in the tail suspension test. The immobility times (mean ± s.e.mean) were 128 ± 16 s for nine controls and 72 ± 12 s for nine SCH 58261-treated mice [$F(2,33)$ 4.61, $P<0.05$].

In another set of experiments, the tail suspension test was carried out in mice which were pre-screened before the assay. Specifically, 140 mice showing the highest immobility time (score ≥ 115 s) considered as 'High-Immobility' animals (III), were selected on day 1 from a sample of 256 tested mice (mean total immobility time: 166 ± 3 s). On the following day, from the 140 III tested mice, 108 mice scored over 115 s (mean total immobility time: 181 ± 4 s). The time of immobility of vehicle-injected III mice on day 3 did not differ significantly from mean scores obtained with the same animals during the screening procedure (i.e., trials 1 and 2). On day 3, mice were injected i.p., 30 min before the test, with either vehicle, SCH 58261 (1, 3, 10 mg kg $^{-1}$, i.p.) or ZM 241385 (15, 30, 60 mg kg $^{-1}$, i.p.). The two adenosine A_{2A} receptor antagonists SCH 58261 and ZM 241385 decreased significantly [$F(6,102)=8.78$, $P<0.001$] the immobility time of screened III animals (Figure 3).

The tail suspension test in selectively bred 'Helpless' mice

SCH 58261 was studied in the tail suspension test using selective bred 'Helpless' CD1 mice. Specifically, experiments were carried out in male and female Swiss albino CD1 'Helpless' mice from the seventh generation of selective breeding for this behavioural trait in our laboratory at the University of Rouen. The reference antidepressant drug imipramine (30 mg kg $^{-1}$, i.p.) reduced by 69% the immobility time [$F(1,42)=96.40$, $P<0.001$]. SCH 58261 also significantly [$F(1,34)=26.80$, $P<0.001$] shortened by 49% the immobility time, i.e. increased struggling time as compared to vehicle-treated animals (Figure 4).

Effects of adenosine A_{2A} receptor antagonists in the forced swim test in CD1 mice

SCH 58261 was administered 30 min before the test at doses ranging from 1 to 10 mg kg $^{-1}$, i.p. The higher dose of 10 mg kg $^{-1}$ reduced the immobility time by 61% (Figure 5A). KW 6002, p.o., decreased the total immobility time at the

doses of 1 and 10 mg kg^{-1}, after oral administration, by 75 and 79%, respectively (Figure 5B).

Role of dopamine D_2 receptors in mediating anti-immobility and stimulant motor effects of acute SCH 58261 in CD1 mice

To assess whether the dopamine D_2 receptors are involved in mediating anti-immobility and stimulant motor effects of SCH 58261, we studied its interaction with haloperidol. Mice received increasing doses of haloperidol (0, 100, 200 μg kg^{-1} s.c.) as a pretreatment 15 min before the administration of an effective dose (10 mg kg^{-1} i.p.) of SCH 58261 in either locomotor activity or forced swim tests. In the locomotor activity test, there was a significant haloperidol-SCH 58261 interaction [$F(2,47)=4.11$, $P<0.05$]. As expected, haloperidol by itself reduced motor activity. However, the stimulant effects of SCH 58261 were not changed by the concomitant presence of haloperidol (Figure 6). In the forced swim test, the two-way ANOVA also showed a significant interaction between the two factors [$F(3,72)=5.04$, $P<0.01$]. Here haloperidol produced no effects over the dose range used (Figure 6, lower panel). However, the effects of SCH 58261 were reversed in the presence of haloperidol (50, 100, 200 μg kg^{-1} i.p.).

The reserpine model in CD1 mice

The vesicular monoamine uptake blocker reserpine (2 mg kg^{-1} s.c.) produced akinesia, hypothermia and ptosis (eye closure). SCH 58261 (3, 10 mg kg^{-1} i.p.), given 210 min after reserpine reversed ptosis but not akinesia nor hypothermia. Specifically, it did not reverse significantly [$F(2,60)=1.64$, $P>0.05$] reserpine-induced akinesia (Table 1). The same animals were also checked for hypothermia and eyelid ptosis before and after the locomotor activity test. Concerning reserpine-induced hypothermia, the effect caused by SCH 58261 did not reach a statistically significant level [$F(2,60)=2.53$, $P=0.08$]. Only eyelid ptosis induced by reserpine was very weakly attenuated, although in a significant [$F(2,60)=9.04$, $P<0.001$] manner, in SCH 58261-treated animals (Table 2).

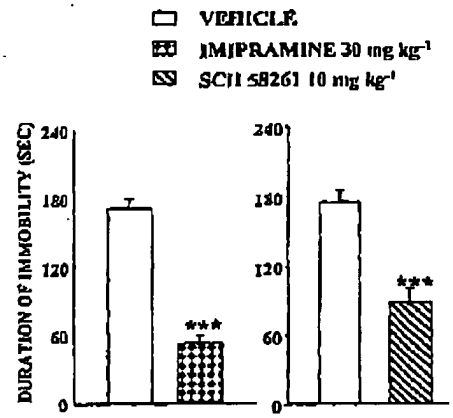

Figure 4 Effects of SCH 58261 or imipramine in the tail suspension test performed in a genetic mouse model of depression. Outbred CD1 mice were used as the foundation population of a line of mice that was selectively bred for its high spontaneous helplessness (immobility scores $\geq$115 s=helpless) in the tail suspension test. Mice of both sexes (7th generation) were injected with SCH 58261 10 mg kg^{-1} i.p. (right panel) or imipramine 30 mg kg^{-1} i.p. (left panel) 30 min before the test. Testing was for 6 min. Means±s.e.mean. of data from 18–22 in each group. ***$P<0.001$ (one-way ANOVA followed by Student-Newman-Keuls test) as compared to vehicle-injected groups.

Discussion

This paper shows that the adenosine A_{2A} receptor may represents a novel target for the discovery of new antidepressants. Specifically, adenosine A_{2A} receptor knockout mice displayed reduction of immobility in functional assays *in vivo*, such as tail suspension and forced swim tests which are predictive of clinical antidepressant activity. Adenosine A_{2A} receptor antagonists were active in the same tests in normal mice.

Adenosine A_{2A} receptor knockout mice were previously found to display reduced locomotor activities in an open field when compared to control mice (Ledent *et al.*, 1997; Chen *et al.*, 1999; El Yacoubi *et al.*, 2000b). Conversely, in the two experimental paradigms used here, the forced swim and the

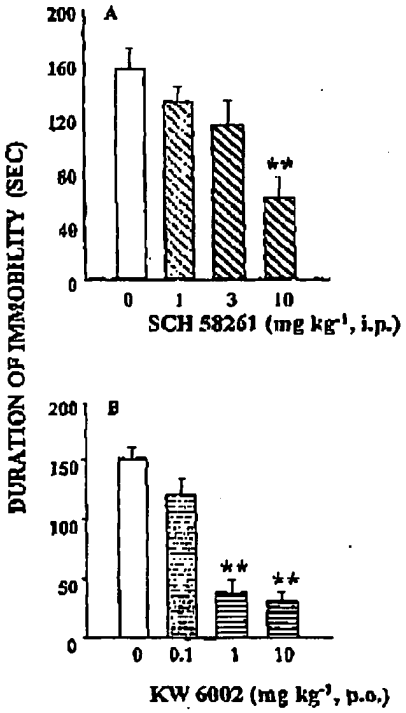

Figure 5 Effects of SCH 58261 (A; 1, 3, 10 mg kg^{-1}, i.p.) and KW 6002 (B; 1, 3, 10 mg kg^{-1}, p.o.) in the mouse forced swim test. Mice were injected with vehicle or SCH 58261 30 min before the test; or received vehicle or KW 6002, 60 min before the test. The duration of immobility was recorded during the last 4-min of the 6-min testing period. Data are mean±s.e.mean. of 10 animals per group. *$P<0.05$, **$P<0.01$ versus vehicle treated group (one-way ANOVA followed by Student-Newman-Keuls test).

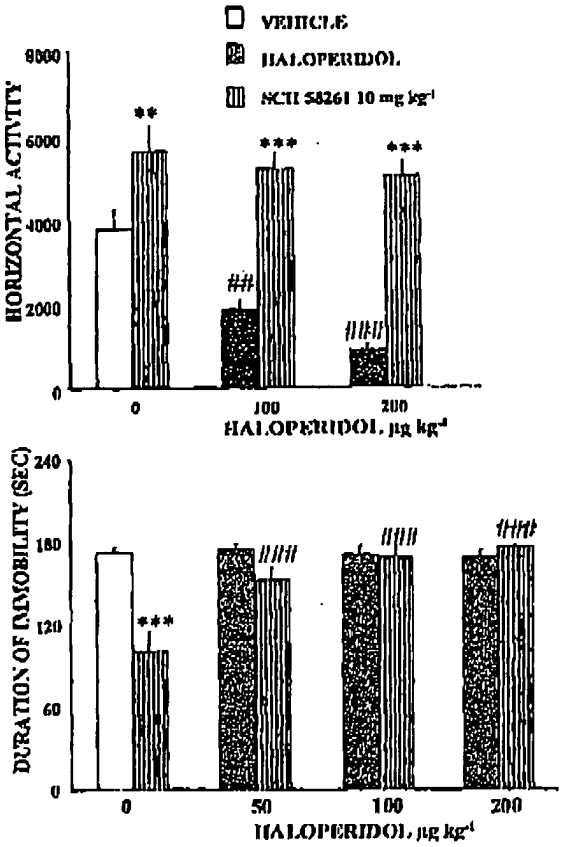

Figure 6 Effects of haloperidol on stimulation of locomotor activity and anti-immobility response induced by SCH 58261. Mice were injected with saline (open bars) or increasing doses of haloperidol (50, 100, 200 µg kg^{-1} i.p.) (hatched bars). Fifteen minutes later, they were injected with vehicle or SCH 58261 (10 mg kg^{-1} i.p.). Upper panel locomotor activity test. Immediately after the second treatment, mice were introduced into the actimeters. The horizontal activity was measured for 45 min. Means ± s.e.mean. of data from 8 mice per group. Two-way ANOVAs: (interaction of haloperidol × SCH 58261): $F(2,47)$ 4.11, P 0.02. Lower panel forced swim test: mice pretreated with haloperidol or saline received vehicle or SCH 58261 30 min before testing. The duration of immobility was recorded during the last 4-min of the 6-min testing period. Means ± s.e.mean of data from 14 controls and 8–11 mice in treated groups. Two-way ANOVAs: (interaction of haloperidol × SCH 58261): $F(3,72)$ 5.04, $P < 0.01$. *Post hoc* comparisons: $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with respective SCH 58261 untreated control groups; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ as compared with respective haloperidol-untreated control groups.

tail suspension tests, their activities were enhanced as compared to those of wildtype mice, suggesting that the neuronal pathways underlying the two behaviours are at least partly different. Reduction of immobility by antidepressants cannot be explained by a non-specific behavioural stimulation as many antidepressants tend to decrease motor activity (Tucker & File, 1986; Perrault *et al.*, 1992). In addition, direct dopamine D_2 receptor agonists, which are known to reduce motor activity when administered in mice (Bouhay *et al.*, 1999), have been shown to increase mobility time in the

Table 1 Effect of the selective A_{2A} receptor antagonist SCH 58261 on motor activity in reserpine-pretreated (2 mg kg^{-1} s.c.) mice

Treatment (mg kg^{-1} i.p.)	*Horizontal activity*
Vehicle	59.38 ± 10.41
SCH 58261 (3)	78.55 ± 20.56
SCH 58261 (10)	127.60 ± 42.38

Mice were injected i.p. with vehicle or SCH 58261 (3, 10 mg kg^{-1} i.p.) 3 h 30 min after a pretreatment with reserpine (2 mg kg^{-1} s.c.) and placed immediately in actimeters. The horizontal component of locomotor activity was measured for 30 min. Data are means ± s.e.mean for 21 controls and 20 mice in treated group. Statistics: No symbol $P > 0.05$ by one-way ANOVA.

forced swim test (Borsini *et al.*, 1988; Duterte-Boucher *et al.*, 1988).

In the tail suspension test, antipsychotics and anxiolytics increase immobility time (Porsolt *et al.*, 1987), whereas adenosine A_{2A} receptor antagonists decrease it. Moreover, adenosine A_{2A} receptor antagonists produce antidepressant-like effects at low doses in comparison to classical antidepressant drugs, such as imipramine and fluoxetine. The efficacy of adenosine A_{2A} receptor antagonists in reducing immobility time in the tail suspension test was confirmed and extended by studies on two different groups of mice. Specifically, the drugs SCH 58261 and ZM 241385 (Ongini *et al.*, 1999; Fredholm & Lindström, 1999), the latter having a lower selectivity profile, were effective in mice previously screened for depressive behaviour (high immobility time). Interestingly, SCH 58261 at 10 mg kg^{-1} reduced immobility in the tail suspension test performed with mice that were selectively bred for their spontaneous 'helplessness' in this test, i.e., a genetic mouse model useful for screening potential antidepressants (Vaugeois *et al.*, 1996). In the same experimental procedure, the tricyclic antidepressant imipramine (30 mg kg^{-1}) induced similar effects. In this study, the effects of repeated administration of SCH 58261 compared to single dose appeared to be attenuated. However, the significance of this result remains doubtful given that data were obtained in different experimental conditions. Nevertheless, if some degree of tolerance to the antidepressant-like effect following chronic treatment with A_{2A} receptor antagonists could be confirmed in future studies, it might be related to an up-regulation of A_{2A} receptor specifically in brain areas implicated in goal-directed behaviours. Since a lack of tolerance to motor stimulant effects of SCH 58261 has been observed in rats (Hallidner *et al.*, 2000), this specific aspect clearly warrants further study.

As part of this pharmacological characterization, SCH 58261 and KW 6002 were also examined in the forced swim test where both drugs reduced the duration of immobility in mice. These results support earlier findings by Sarges *et al.* (1990) showing that a weakly selective A_{2A} receptor antagonist, CP 66, 713 (25 fold selectivity A_{2A} vs A_1), was effective in the forced swim test.

Additional experiments were carried out with the more selective compound SCH 58261. An interaction study with the dopamine D_2 receptor antagonist (Seeman, 1980) haloperidol was performed with the aim to discriminate

Table 2 Effect of the selective A_2 receptor antagonist SCH 58261 on reserpine-induced (2 mg kg^{-1} s.c.) eyelid ptosis and hypothermia in mice

Treatment (mg kg^{-1} i.p.)	*Ptosis before treatment (PB)*	*Ptosis after treatment (PA)*	*PB-PA*
Vehicle	6.90±0.18	7.43±0.16	−0.52±0.19
SCH 58261 (3)	7.55±0.15	6.90±0.22	0.65±0.22**
SCH 58261 (10)	7.15±0.17	6.85±0.15	0.30±0.19**
	Temperature before treatment (TB)	*Temperature after treatment (TA)*	*TB-TA*
Vehicle	33.79±0.37	31.23±0.49	2.56±0.33
SCH 58261 (3)	34.19±0.33	32.48±0.33	1.71±0.26
SCH 58261 (10)	33.97±0.27	31.50±0.39	2.47±0.29

Mice were injected with vehicle or SCH 58261 (3, 10 mg kg^{-1} i.p.) 3 h 30 min after pretreatment with reserpine (2 mg kg^{-1} s.c.). Ptosis and temperature were assessed just before treatment and 30 min later. Means±s.e.mean of data from 20 mice per group. Statistics: No symbol $P>0.05$, **$P<0.01$, ***$P<0.001$ as compared to vehicle using one-way ANOVA followed by Student-Newman-Keuls test).

an escape-directed behaviour (i.e. a loss of motivation to avoid the stressful situation) from its motor stimulant effects (Svenningsson *et al.*, 1997b; Popoli *et al.*, 1998; El Yacoubi *et al.*, 2000c). Here the anti-immobility effect elicited by SCH 58261 was prevented by a low dose (0.05 mg kg^{-1}) of the dopamine D_2 receptor antagonist, demonstrating a high sensitivity of the goal-directed behaviour to haloperidol. It is worth comparing this finding with those of other studies showing that dopamine D_2 receptor antagonists block anti-immobility effects of antidepressants (Borsini *et al.*, 1985; Borsini & Meli, 1990). SCH 58261-induced stimulant motor effects were not counteracted by haloperidol administered at moderate doses (0.1–0.2 mg kg^{-1}) used in the present work. Moreover, adenosine A_{2A} receptor antagonists effectively reduce catalepsy induced by high doses (in the mg kg^{-1} range) of dopamine D_2 antagonists, a screening test for potential antiparkinsonian drugs (Kanda *et al.*, 1994; Kafka & Corbett, 1996). Altogether, these data suggest that targeting with drugs the dopamine D_2 and adenosine A_{2A} receptors may result in swings in opposite directions of the physiological balance that exists between the neurotransmitter and neuromodulator, depending on the neuronal systems implicated in a particular function.

Therefore, dopamine transmission through dopamine D_2 receptors appears to be critically involved in the anti-immobility effect elicited by SCH 58261. It has been suggested that disturbances in dopamine transmission are involved in the pathophysiology of mood disorders. For example, the antidepressant bupropion is a dopamine and noradrenaline reuptake inhibitor that has a direct enhancing action upon dopamine transmission (Cooper *et al.*, 1980; Davidson & Connor, 1998), whereas a depressive syndrome is frequently encountered in subjects affected by Parkinson's disease, where dopamine depletion is observed (Allain *et al.*, 2000). Dopamine transmission in both frontal cortex and nucleus accumbens has been implicated in the mechanism of action of antidepressants (Tanda *et al.*, 1994; Fibiger, 1995). One tentative explanation for the dissociation between the two behaviours studied here may reside in the peculiar physiology and pharmacology of dopamine neurones originating in the ventral tegmental area in the midbrain and projecting to the prefrontal cortex. They are known to have higher turnover rates and enhanced burst firing compared to mesolimbic and nigrostriatal dopamine neurones, and to have high responsiveness to mild stressors (Deutch & Roth, 1990). In contrast to nigrostriatal dopamine neurones, mesoprefrontal dopamine neurones may also not be well suited for maintaining homeostasis due to the absence or low sensitivity of synthesis- and impulse-regulating autoreceptors (Deutch & Roth, 1990). Due to the blockade of synthesis- and impulse-regulating autoreceptors projecting to dorsal and ventral striatum, haloperidol by itself induces release of dopamine to reach a synaptic concentration allowing a competition with haloperidol, which could explain, in part, the lack of antagonism of SCH 58261-induced effects in the motor activity test. On the contrary, dopamine release elicited by haloperidol in the frontal cortex would be much weaker in intensity (Moghaddam & Bunney, 1990), allowing the reversal of the anti-immobility effect caused by the selective adenosine A_{2A} receptor antagonist. This hypothesis is supported by the evidence that antidepressants mostly increase extracellular dopamine release in the frontal cortex than in the nucleus accumbens (Tanda *et al.*, 1994).

The adenosine A_{2A} receptor has been visualized by autoradiography in the prefrontal cortex of the mouse (Johansson *et al.*, 1996) and rat (Ishiwata *et al.*, 2000) with densities equal respectively to about one tenth and one fifth that found in the striatum. The selective adenosine A_{2A} receptor antagonist [^{3}H] SCH 58261 was also found to label the postmortem human prefrontal cortex, with a binding density about one third that detected in rostral putamen (Svenningsson *et al.*, 1997a). A role of adenosine A_{2A} receptor located in the striatum cannot be completely excluded, since dopamine transmission in this structure plays an important role in determining the individual flexibility to cope with available sensory information (Cools, 1980), and dopamine D_2 receptor densities are modified in striatum of depressed patients relative to controls (D'Haenen & Bossuyt, 1994; Shah *et al.*, 1997).

The stimulant motor effects elicited by SCH 58261 in reserpine-pretreated mice were mild in our experimental conditions. Shiozaki *et al.* (1999) have reported a reversal of reserpine-induced akinesia by another adenosine A_{2A} receptor antagonist. Further work will be necessary to explain the lack of reserpine-induced akinesia in the present study. The absence of effects upon eyelid ptosis and hypothermia induced by reserpine suggests that adenosine

A_{2A} receptors are not involved in the modulation of noradrenergic neuronal pathways underlying these behaviours (Bourin *et al.*, 1983).

The models of depression that we have used have however some limitations. As previously mentioned by several authors (Willner, 1990; Weiss & Kilts, 1998), one of the major drawbacks of the forced swim and tail suspension tests is the positive response elicited after an acute administration of antidepressants. Although it is widely accepted that these tests are useful to screen potential antidepressants, selective A_{2A} receptor antagonists should be further examined both in other preclinical models such as learned helplessness or chronic mild stress and after repeated treatments. It is also worth noting that useful and robust information can only emerge when selective adenosine A_{2A} receptor antagonists will be studied in patients such as those affected by Parkinson's disease.

In conclusion, these data support the hypothesis that adenosine A_{2A} receptor antagonists enhance the activity of mice in the forced swim and tail suspension tests by a prolongation of escape-directed behaviour, rather than by a generalized motor stimulant effect. The positive effect is likely mediated by an increase in dopaminergic transmission, possibly in frontal cortex. Modulation of monoamine activity as a therapeutic strategy dominates antidepressant research. However, all antidepressants developed so far exert their therapeutic effects with an undesirable delay, and there is still a need to fill this therapeutic gap. In this sense, adenosine A_{2A} receptor antagonists might offer a novel approach to the treatment of depression.

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